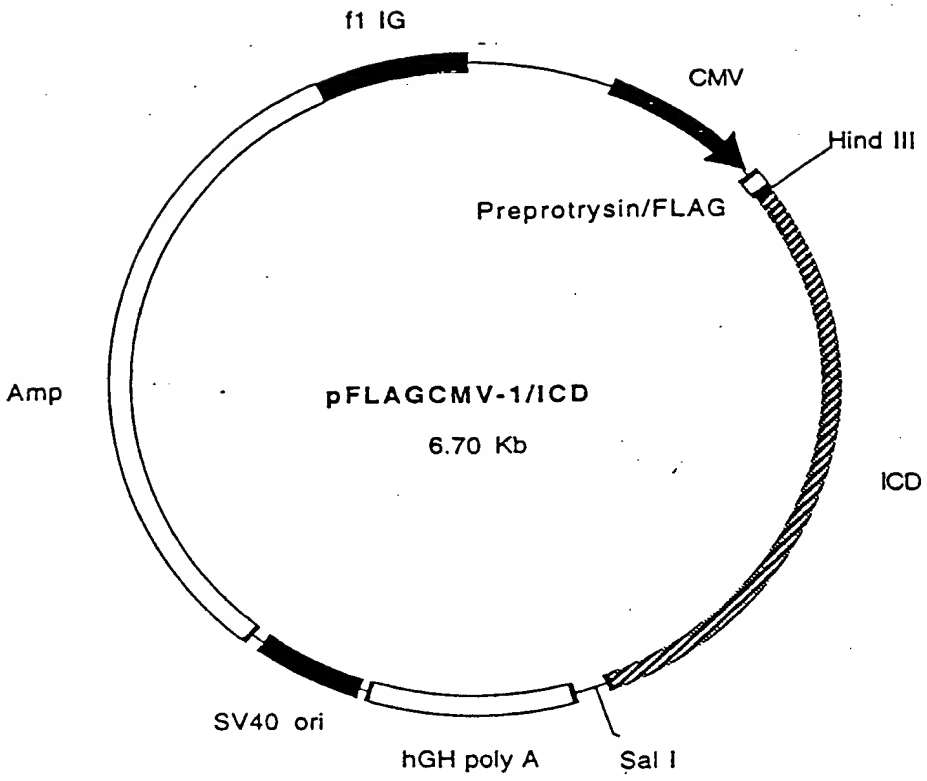
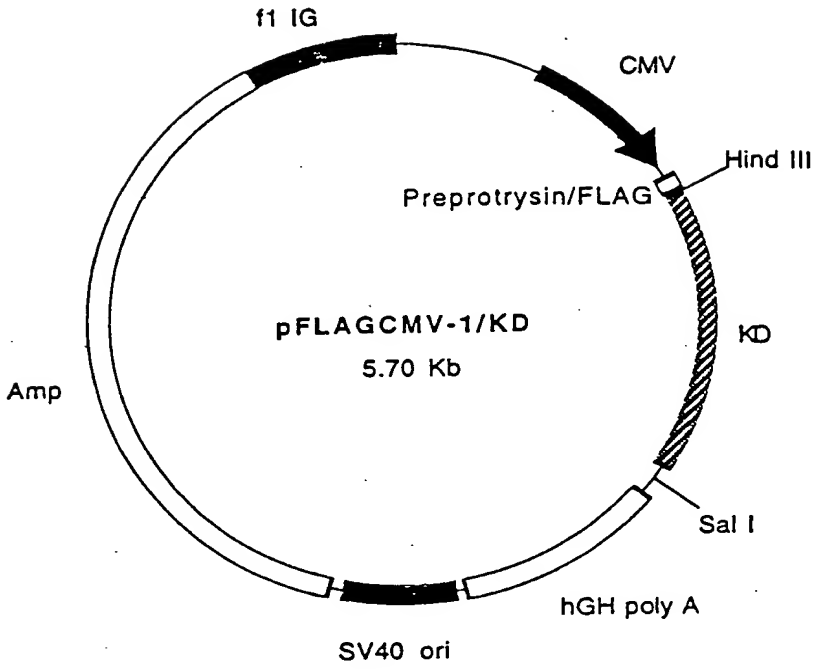

f1 IG
CMV
Hind III
Preprotrysin/FLAG
Amp
pFLAGCMV-1/ICD
6.70 Kb
ICD
SV40 ori
hGH poly A
Sal I

Fig. 1

Fig. 2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Hind III
Preprotrysin/FLAG
pFLAGCMV-1/KD
5.70 Kb
KD
Amp
Sal I
hGH poly A
SV40 ori

Fig. 3

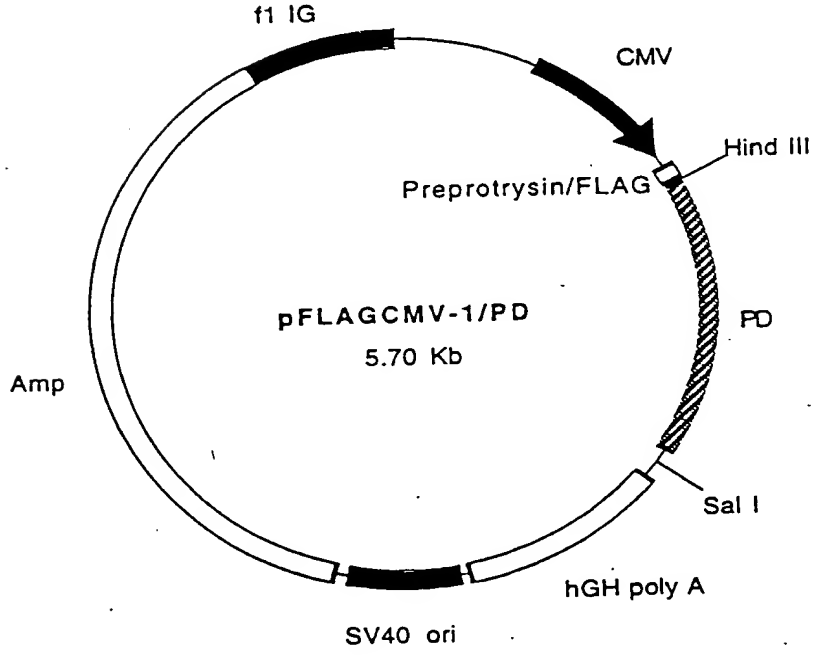
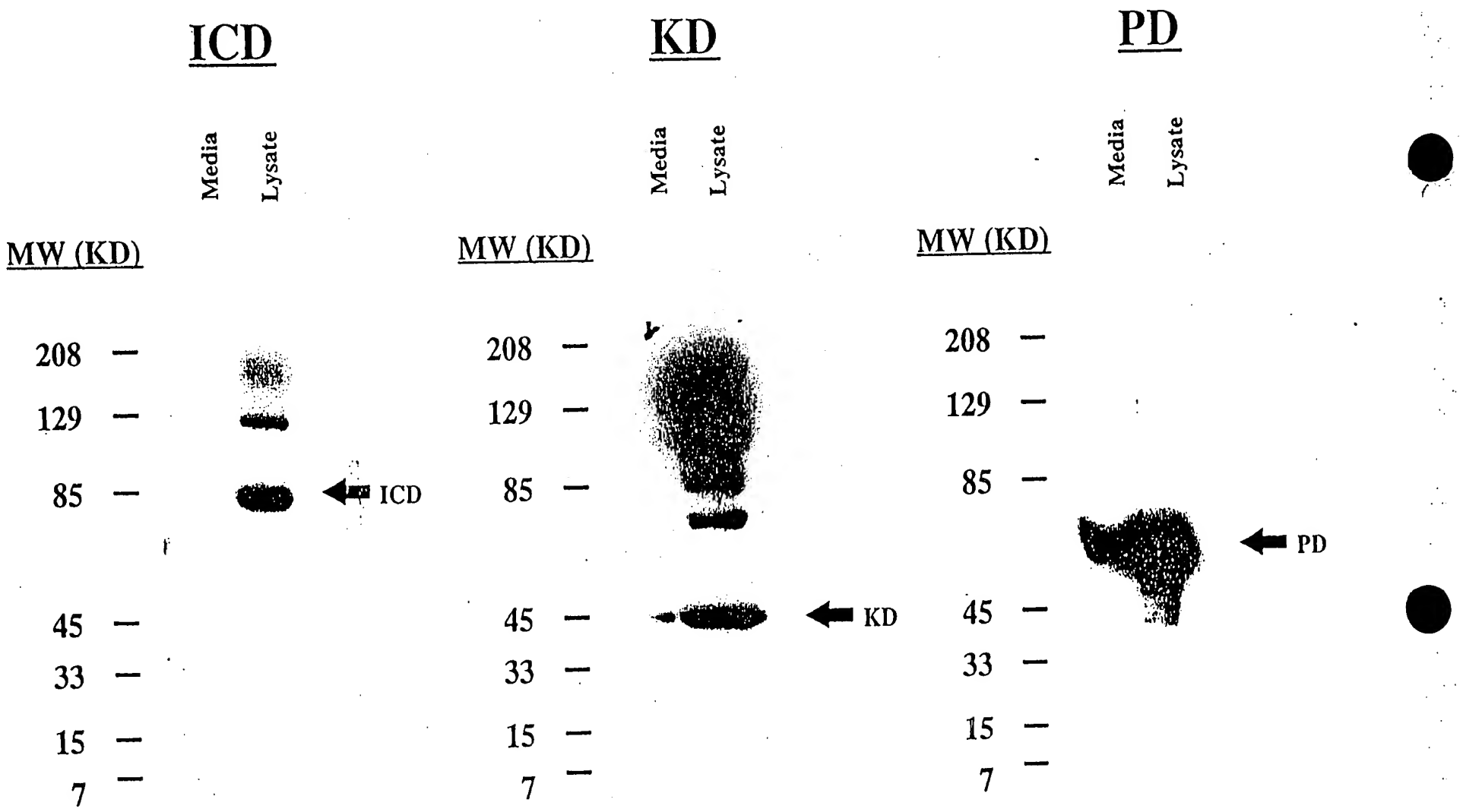

f1 IG
CMV
Hind III
Preprotrysin/FLAG
pFLAGCMV-1/PD
5.70 Kb
PD
Amp
Sal I
hGH poly A
SV40 ori

Fig. 4



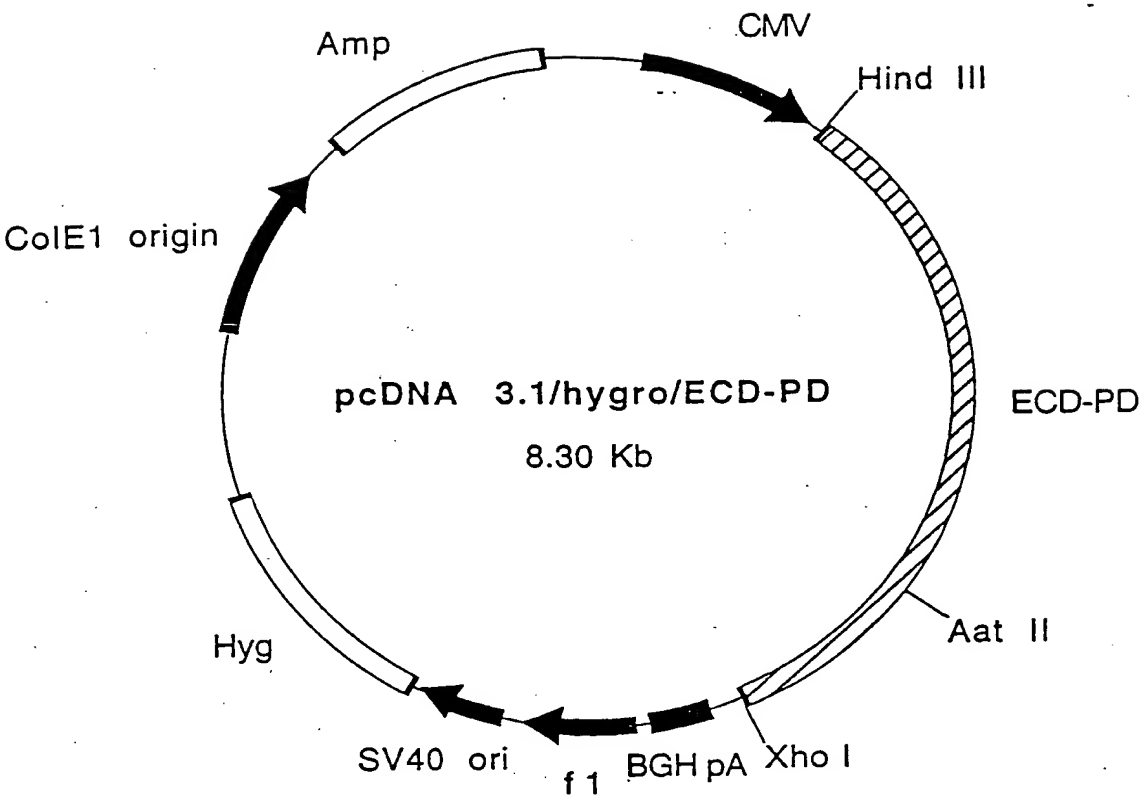

Amp
CMV
Hind III
ColE1 origin
pcDNA 3.1/hygro/ECD-PD
8.30 Kb
ECD-PD
Hyg
Aat II
SV40 ori
f1
BGH pA
Xho I

Fig. 5

pcDNA3.1hyg/ECD-PD expression

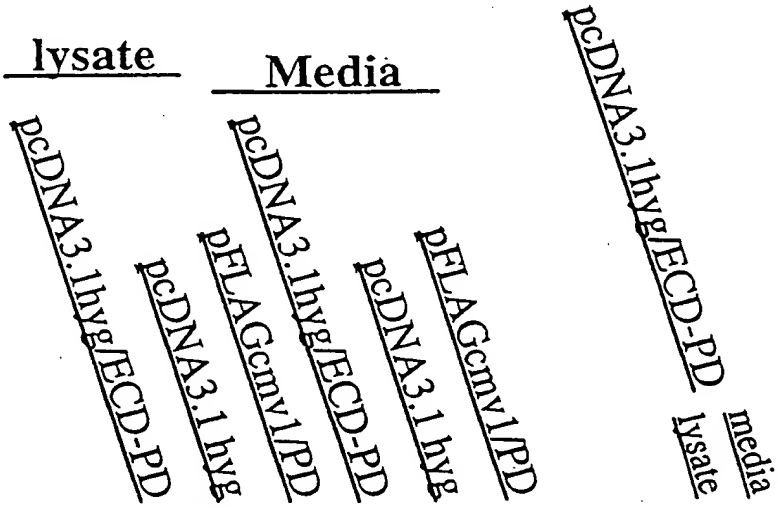


Fig. 6A

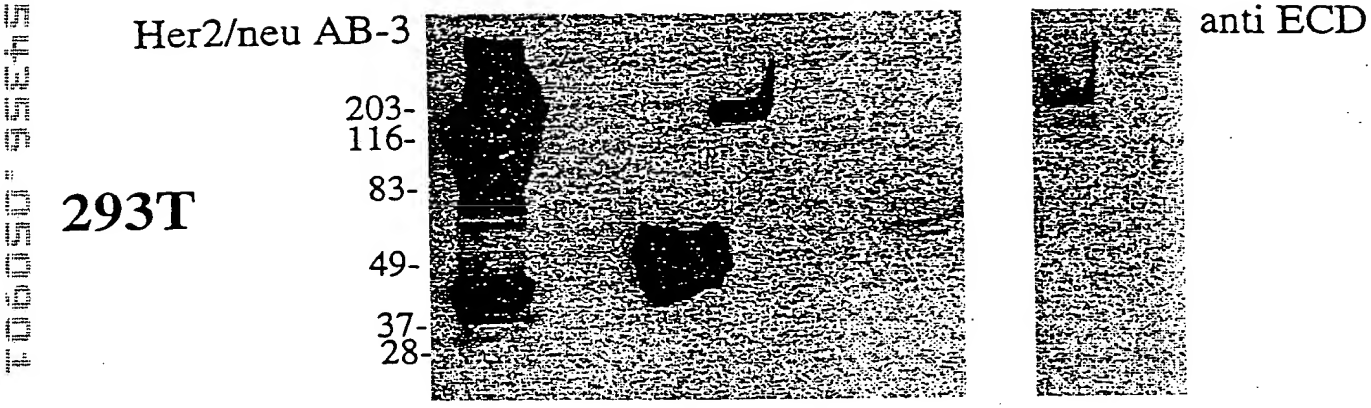


Fig. 6B

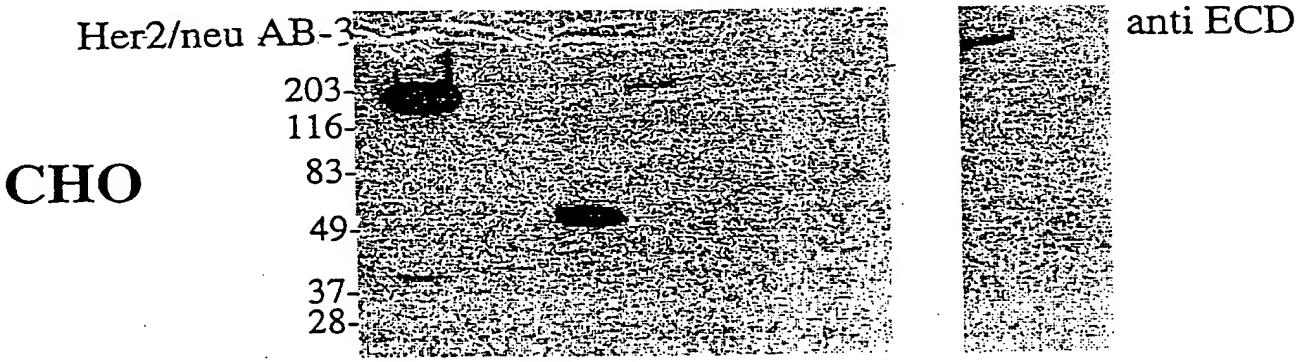


Fig. 7 (SEQ ID NO: 1)

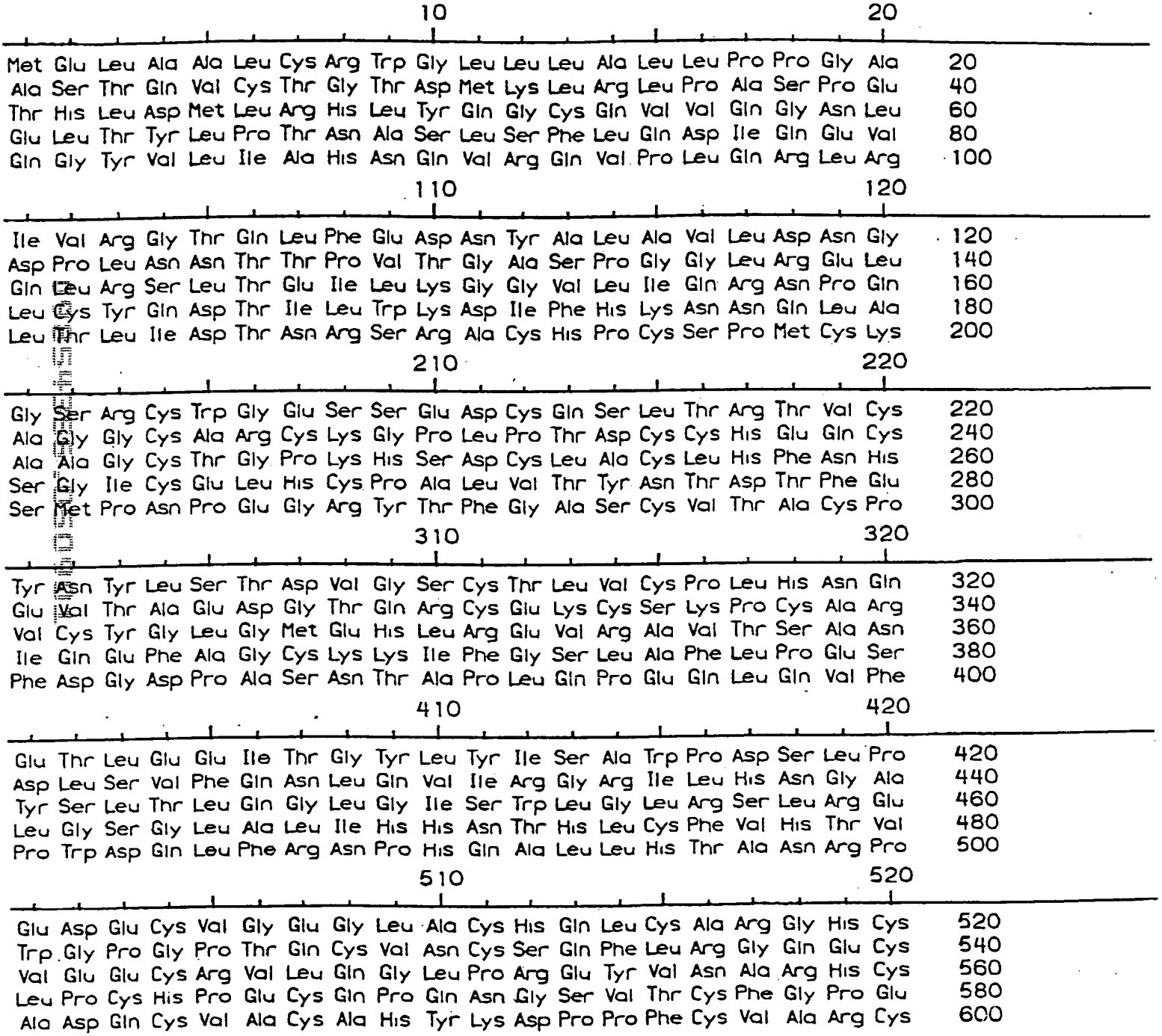


Fig. 7 (SEQ ID NO: 1)

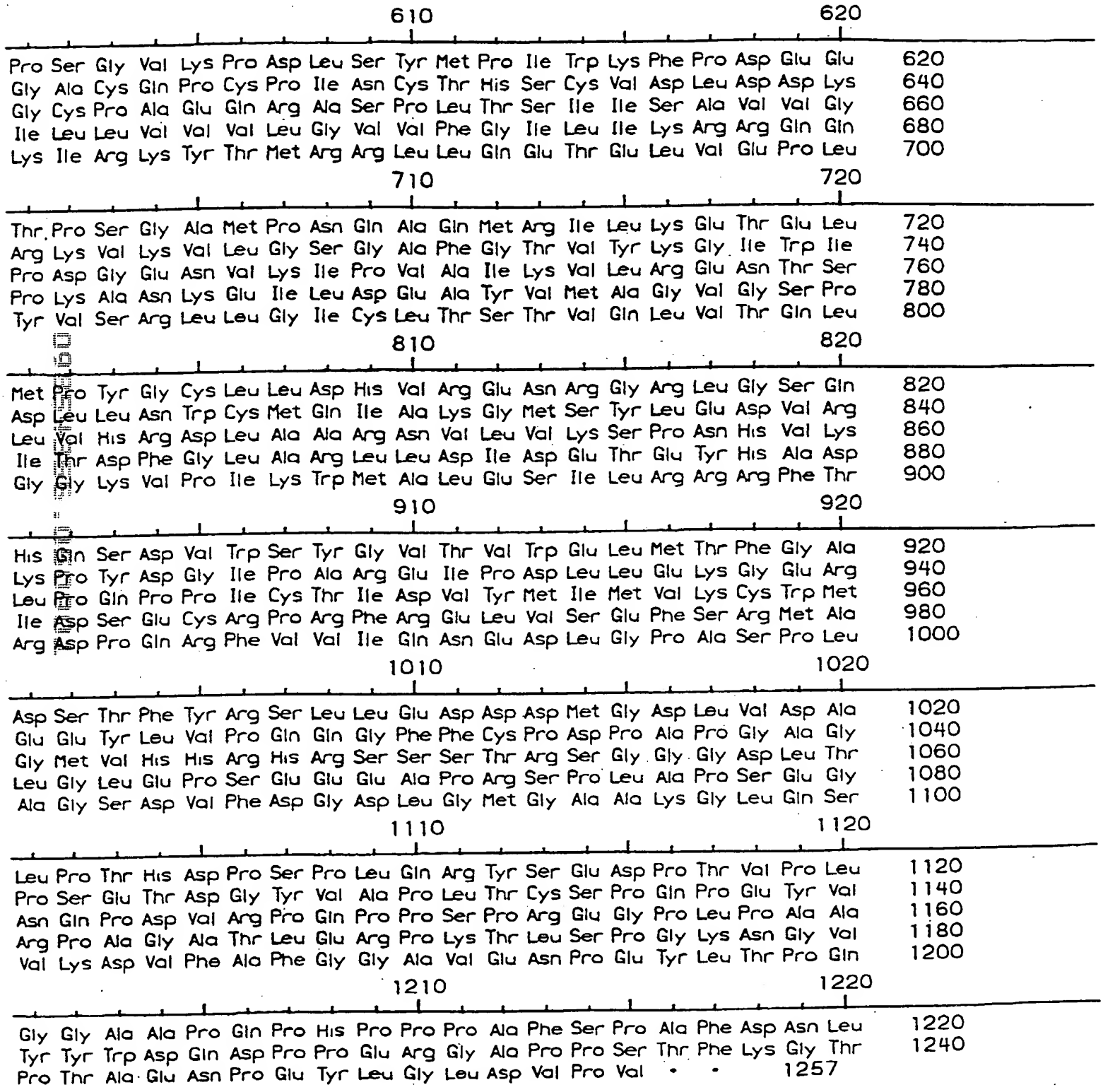


Fig. 8 (SEQ ID NO: 2)

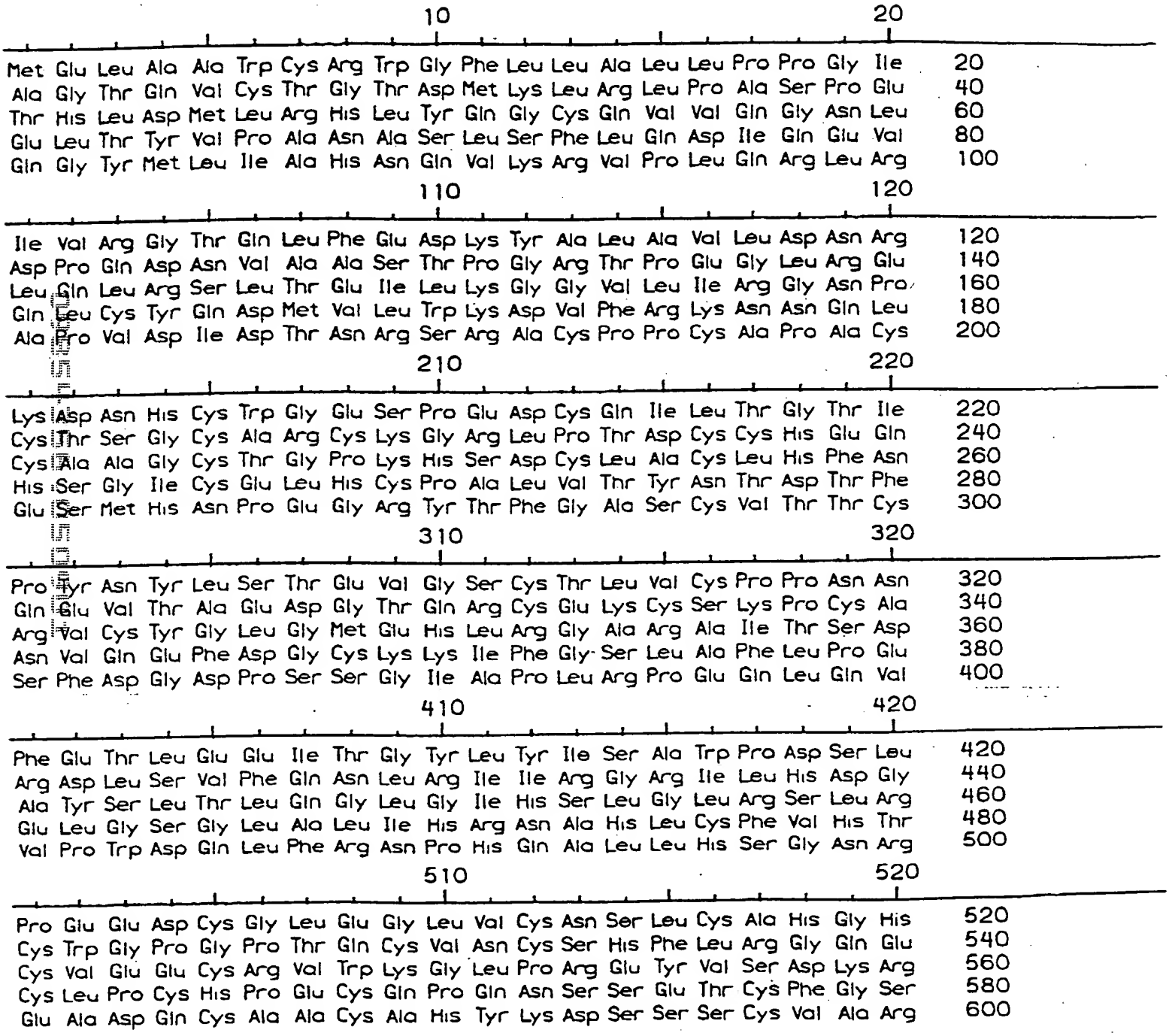


Fig. 8 (SEQ ID NO: 2)

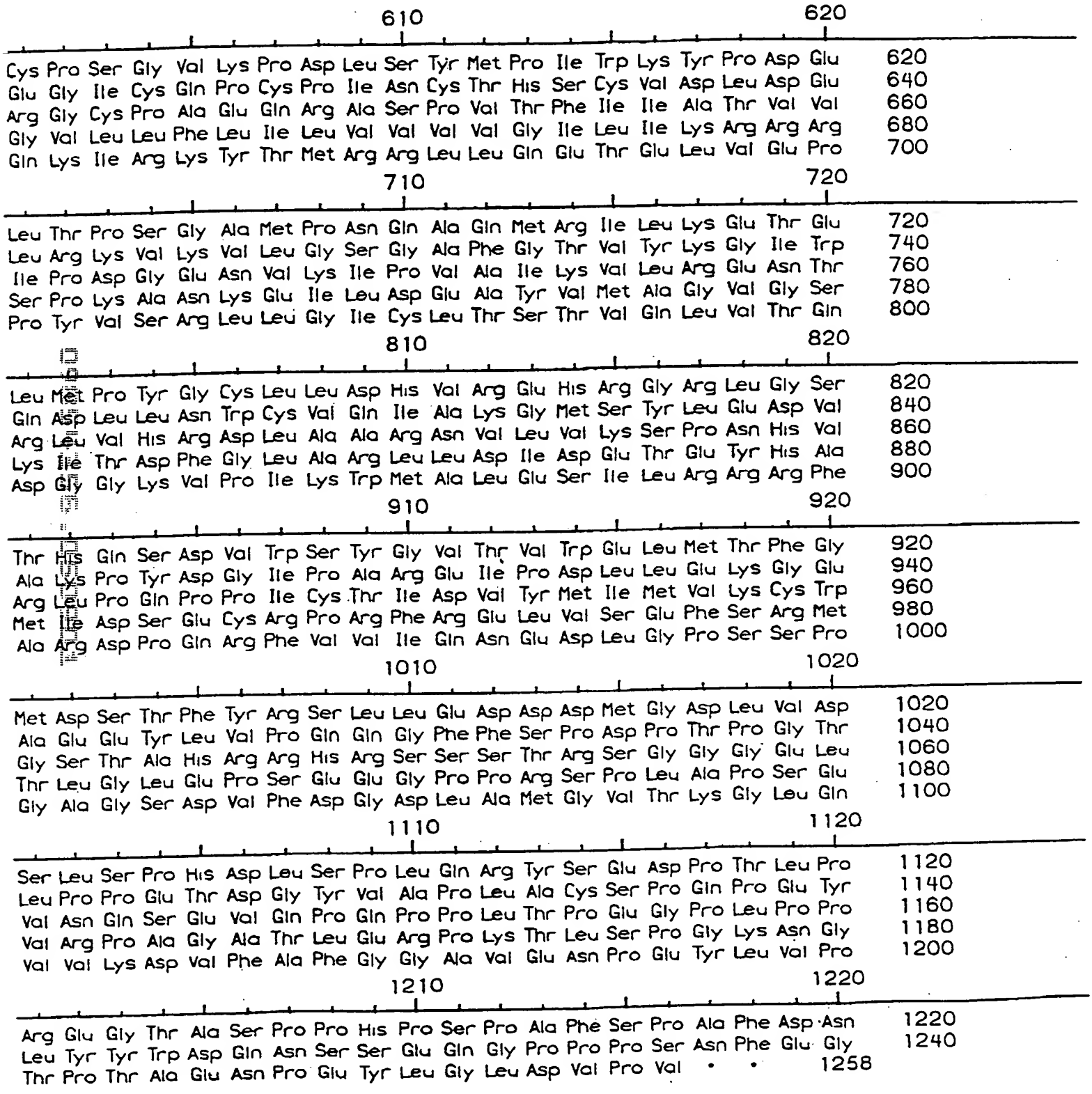
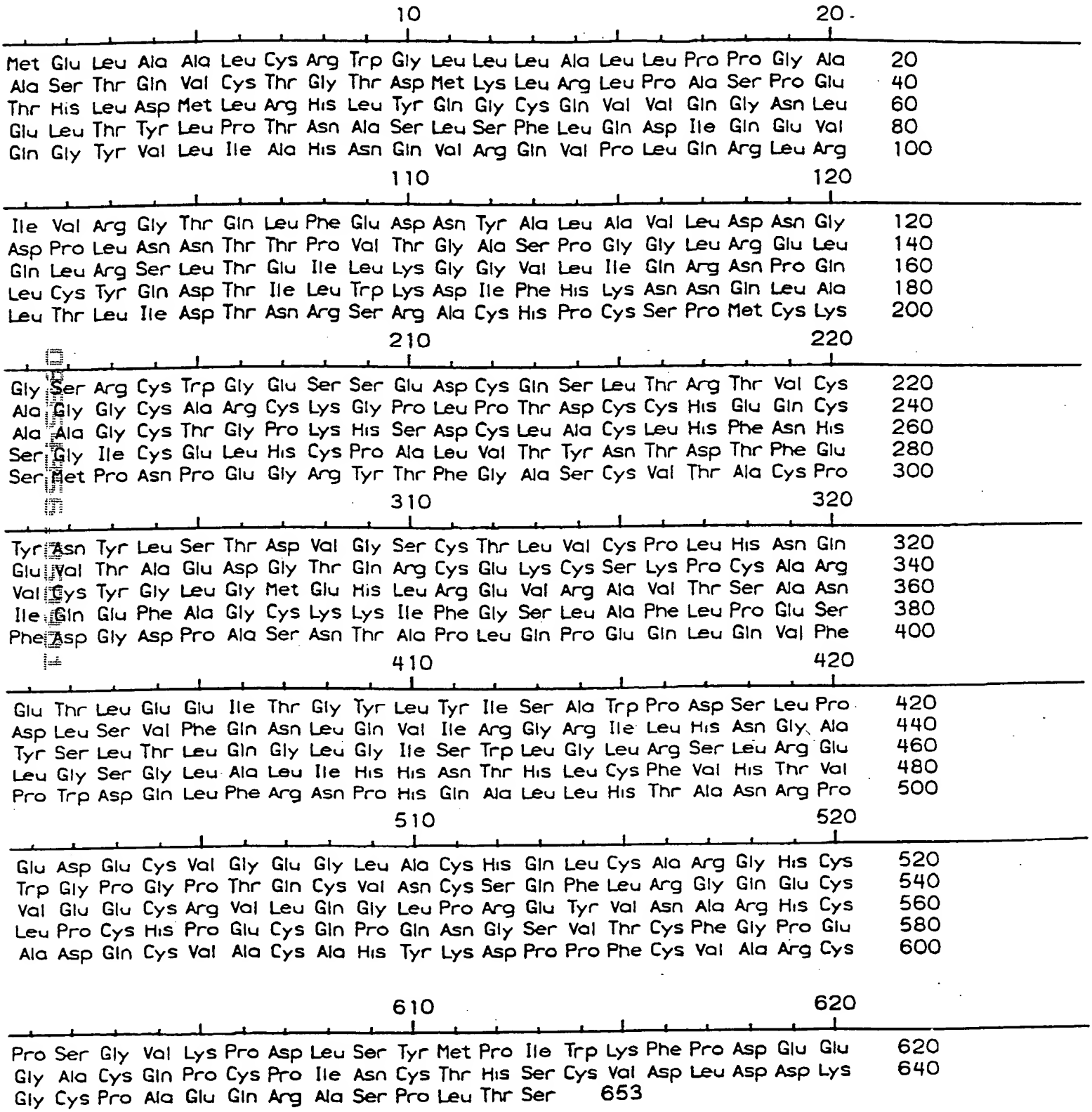


Fig. 9 (SEQ ID NO: 3)



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                                        10                  20
Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu Pro Pro Gly Ala    20
Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys Leu Arg Leu Pro Ala Ser Pro Glu    40
Thr His Leu Asp Met Leu Arg His Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu    60
Glu Leu Thr Tyr Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val    80
Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu Gln Arg Leu Arg   100
                                       110                 120
Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr Ala Leu Ala Val Leu Asp Asn Gly   120
Asp Pro Leu Asn Asn Thr Thr Pro Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu   140
Gln Leu Arg Ser Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln   160
Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn Asn Gln Leu Ala   180
Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys His Pro Cys Ser Pro Met Cys Lys   200
                                       210                 220
Gly Ser Arg Cys Trp Gly Glu Ser Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys   220
Ala Gly Gly Cys Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys   240
Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu His Phe Asn His   260
Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val Thr Tyr Asn Thr Asp Thr Phe Glu   280
Ser Met Pro Asn Pro Glu Gly Arg Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro   300
                                       310                 320
Tyr Asn Tyr Leu Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln   320
Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys Pro Cys Ala Arg   340
Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu Val Arg Ala Val Thr Ser Ala Asn   360
Ile Gln Glu Phe Ala Gly Cys Lys Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser   380
Phe Asp Gly Asp Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe   400
                                       410                 420
Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro Asp Ser Leu Pro   420
Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg Gly Arg Ile Leu His Asn Gly Ala   440
Tyr Ser Leu Thr Leu Gln Gly Leu Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu   460
Leu Gly Ser Gly Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val   480
Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr Ala Asn Arg Pro   500
                                       510                 520
Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His Gln Leu Cys Ala Arg Gly His Cys   520
Trp Gly Pro Gly Pro Thr Gln Cys Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys   540
Val Glu Glu Cys Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys   560
Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys Phe Gly Pro Glu   580
Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp Pro Pro Phe Cys Val Ala Arg Cys   600
                                       610                 620
Pro Ser Gly Val Lys Pro Asp Leu Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu   620
Gly Ala Cys Gln Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys   640
Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser  653
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Fig. 10 (SEQ ID NO: 4)

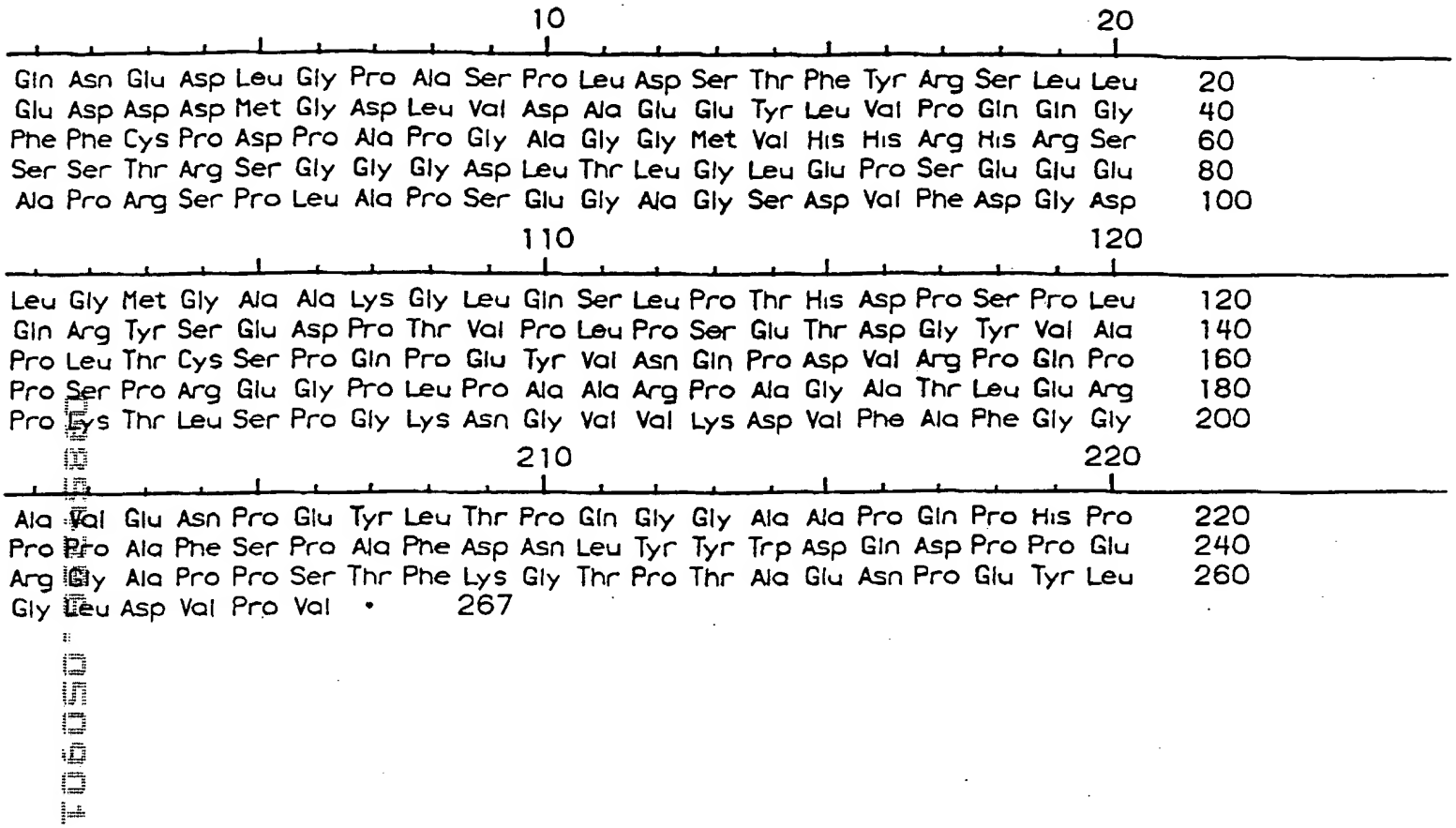

Fig. 11 (SEQ ID NO: 5)

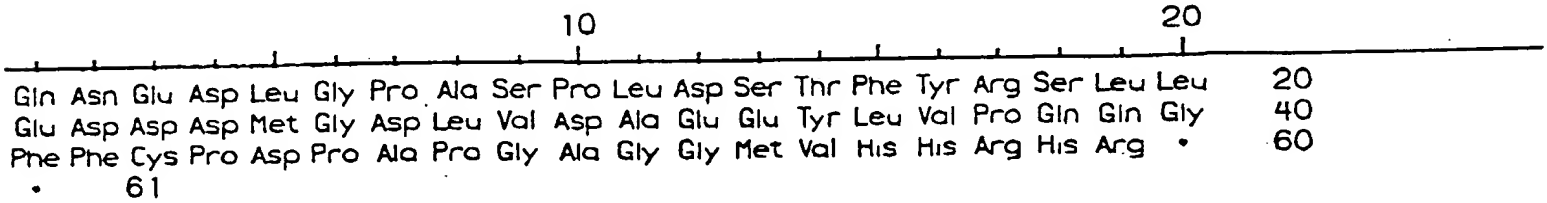

Fig. 12 (SEQ ID NO: 6)

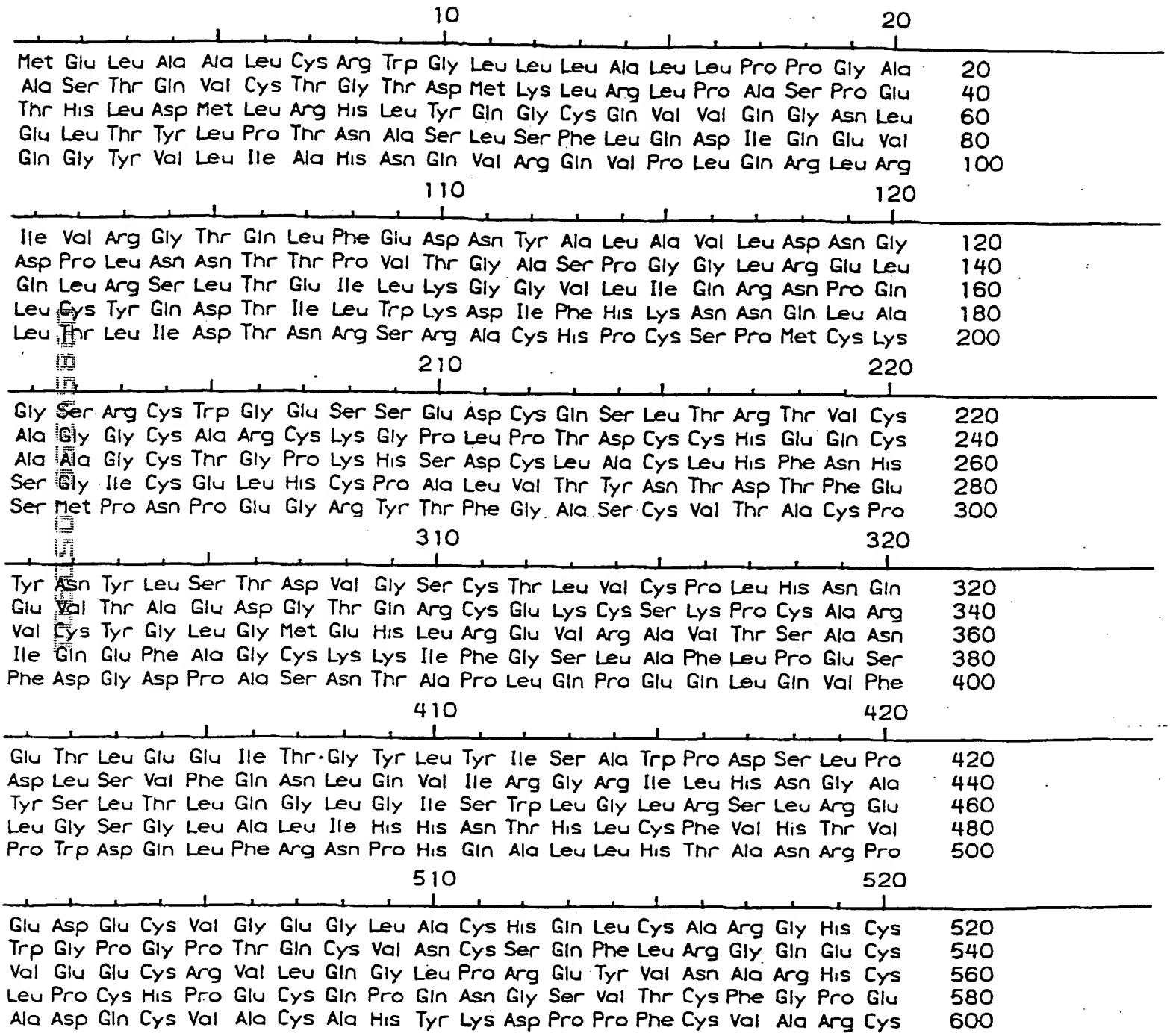

Fig. 12 (SEQ ID NO: 6)

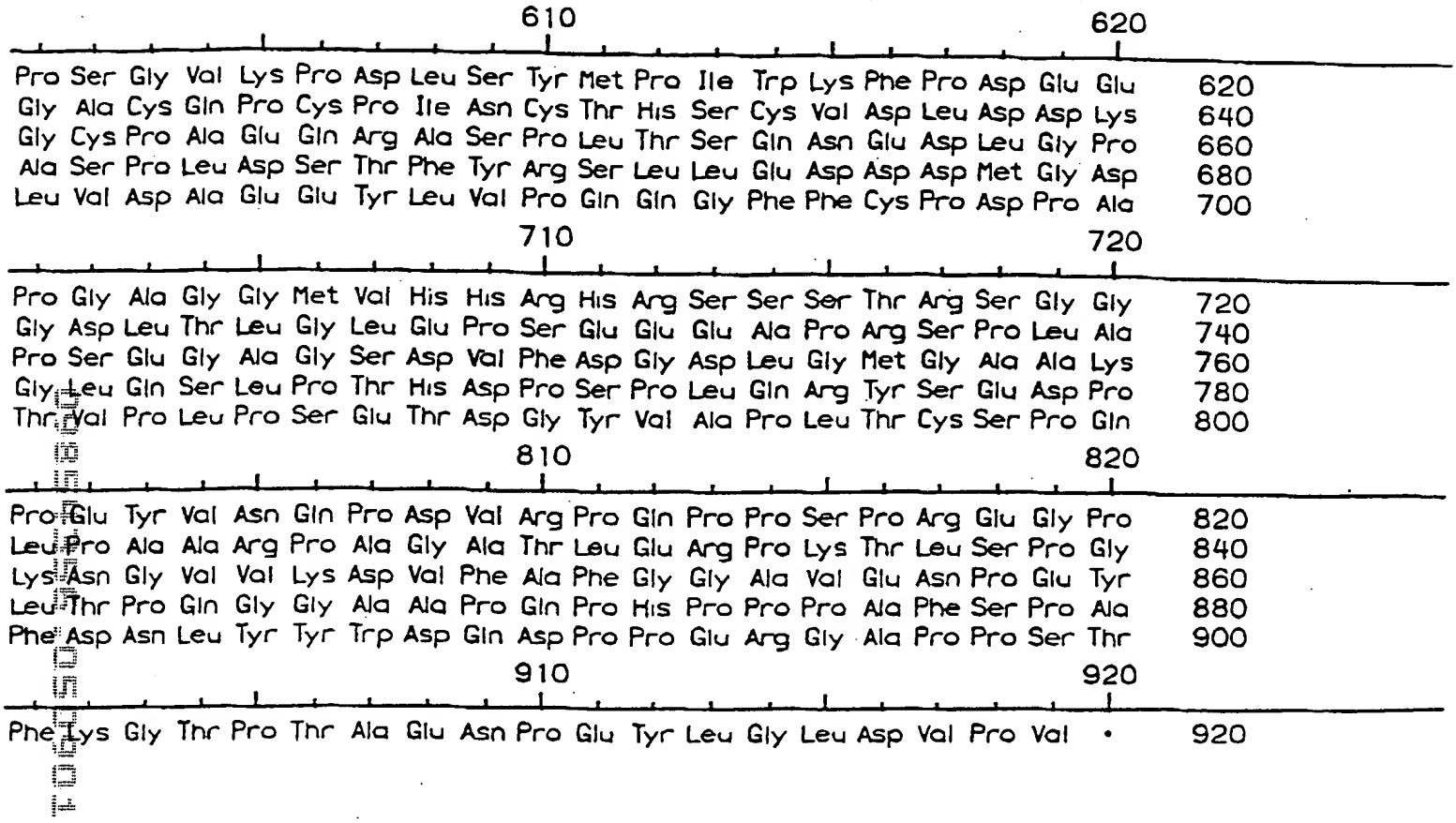

Fig. 13 (SEQ ID NO: 7)

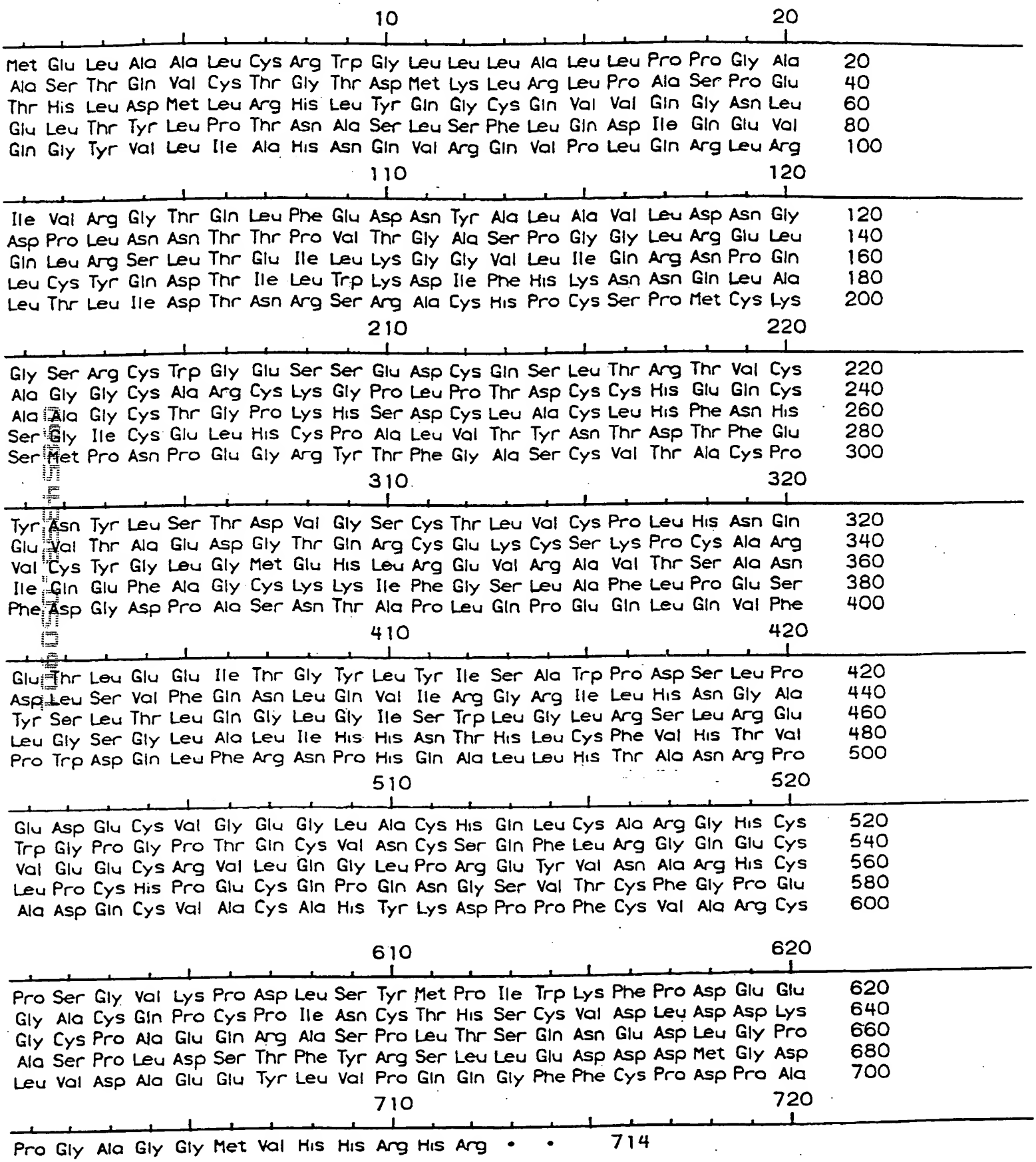

Fig. 14 (SEQ ID NO: 8)

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                                        10                                  20
Met Glu Leu Ala Ala Trp Cys Arg Trp Gly Phe Leu Leu Ala Leu Leu Pro Pro Gly Ile    20
Ala Gly Thr Gln Val Cys Thr Gly Thr Asp Met Lys Leu Arg Leu Pro Ala Ser Pro Glu    40
Thr His Leu Asp Met Leu Arg His Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu    60
Glu Leu Thr Tyr Val Pro Ala Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val    80
Gln Gly Tyr Met Leu Ile Ala His Asn Gln Val Lys Arg Val Pro Leu Gln Arg Leu Arg   100
                                       110                                 120
Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Lys Tyr Ala Leu Ala Val Leu Asp Asn Arg   120
Asp Pro Gln Asp Asn Val Ala Ala Ser Thr Pro Gly Arg Thr Pro Glu Gly Leu Arg Glu   140
Leu Gln Leu Arg Ser Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Arg Gly Asn Pro   160
Gln Leu Cys Tyr Gln Asp Met Val Leu Trp Lys Asp Val Phe Arg Lys Asn Asn Gln Leu   180
Ala Pro Val Asp Ile Asp Thr Asn Arg Ser Arg Ala Cys Pro Pro Cys Ala Pro Ala Cys   200
                                       210                                 220
Lys Asp Asn His Cys Trp Gly Glu Ser Pro Glu Asp Cys Gln Ile Leu Thr Gly Thr Ile   220
Cys Thr Ser Gly Cys Ala Arg Cys Lys Gly Arg Leu Pro Thr Asp Cys Cys His Glu Gln   240
Cys Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu His Phe Asn   260
His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val Thr Tyr Asn Thr Asp Thr Phe   280
Glu Ser Met His Asn Pro Glu Gly Arg Tyr Thr Phe Gly Ala Ser Cys Val Thr Thr Cys   300
                                       310                                 320
Pro Tyr Asn Tyr Leu Ser Thr Glu Val Gly Ser Cys Thr Leu Val Cys Pro Pro Asn Asn   320
Gln Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys Pro Cys Ala   340
Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Gly Ala Arg Ala Ile Thr Ser Asp   360
Asn Val Gln Glu Phe Asp Gly Cys Lys Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu   380
Ser Phe Asp Gly Asp Pro Ser Ser Gly Ile Ala Pro Leu Arg Pro Glu Gln Leu Gln Val   400
                                       410                                 420
Phe Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro Asp Ser Leu   420
Arg Asp Leu Ser Val Phe Gln Asn Leu Arg Ile Ile Arg Gly Arg Ile Leu His Asp Gly   440
Ala Tyr Ser Leu Thr Leu Gln Gly Leu Gly Ile His Ser Leu Gly Leu Arg Ser Leu Arg   460
Glu Leu Gly Ser Gly Leu Ala Leu Ile His Arg Asn Ala His Leu Cys Phe Val His Thr   480
Val Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Ser Gly Asn Arg   500
                                       510                                 520
Pro Glu Glu Asp Cys Gly Leu Glu Gly Leu Val Cys Asn Ser Leu Cys Ala His Gly His   520
Cys Trp Gly Pro Gly Pro Thr Gln Cys Val Asn Cys Ser His Phe Leu Arg Gly Gln Glu   540
Cys Val Glu Glu Cys Arg Val Trp Lys Gly Leu Pro Arg Glu Tyr Val Ser Asp Lys Arg   560
Cys Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Ser Ser Glu Thr Cys Phe Gly Ser   580
Glu Ala Asp Gln Cys Ala Ala Cys Ala His Tyr Lys Asp Ser Ser Ser Cys Val Ala Arg   600
                                       610                                 620
Cys Pro Ser Gly Val Lys Pro Asp Leu Ser Tyr Met Pro Ile Trp Lys Tyr Pro Asp Glu   620
Glu Gly Ile Cys Gln Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Glu   640
Arg Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Val Thr Phe    654
```

Fig. 15 (SEQ ID NO: 9)

```
ATG GAG CTG GCG GCC TTG TGC CGC TGG GGG CTC CTC CTC GCC CTC TTG          48
Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu
 1               5                  10                  15

CCC CCC GGA GCC GCG AGC ACC CAA GTG TGC ACC GGC ACA GAC ATG AAG          96
Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
            20                  25                  30

CTG CGG CTC CCT GCC AGT CCC GAG ACC CAC CTG GAC ATG CTC CGC CAC         144
Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
        35                  40                  45

CTC TAC CAG GGC TGC CAG GTG GTG CAG GGA AAC CTG GAA CTC ACC TAC         192
Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
    50                  55                  60

CTG CCC ACC AAT GCC AGC CTG TCC TTC CTG CAG GAT ATC CAG GAG GTG         240
Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65                  70                  75                  80

CAG GGC TAC GTG CTC ATC GCT CAC AAC CAA GTG AGG CAG GTC CCA CTG         288
Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
                85                  90                  95

CAG AGG CTG CGG ATT GTG CGA GGC ACC CAG CTC TTT GAG GAC AAC TAT         336
Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
            100                 105                 110

GCC CTG GCC GTG CTA GAC AAT GGA GAC CCG CTG AAC AAT ACC ACC CCT         384
Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
        115                 120                 125
```

Fig. 15 (SEQ ID NO: 9)

```
GTC ACA GGG GCC TCC CCA GGA GGC CTG CGG GAG CTG CAG CTT CGA AGC     432
Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
    130                 135                 140

CTC ACA GAG ATC TTG AAA GGA GGG GTC TTG ATC CAG CGG AAC CCC CAG     480
Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
145                 150                 155                 160

CTC TGC TAC CAG GAC ACG ATT TTG TGG AAG GAC ATC TTC CAC AAG AAC     528
Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
                165                 170                 175

AAC CAG CTG GCT CTC ACA CTG ATA GAC ACC AAC CGC TCT CGG GCC TGC     576
Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
            180                 185                 190

CAC CCC TGT TCT CCG ATG TGT AAG GGC TCC CGC TGC TGG GGA GAG AGT     624
His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
        195                 200                 205

TCT GAG GAT TGT CAG AGC CTG ACG CGC ACT GTC TGT GCC GGT GGC TGT     672
Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
    210                 215                 220

GCC CGC TGC AAG GGG CCA CTG CCC ACT GAC TGC TGC CAT GAG CAG TGT     720
Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
225                 230                 235                 240

GCT GCC GGC TGC ACG GGC CCC AAG CAC TCT GAC TGC CTG GCC TGC CTC     768
Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
                245                 250                 255

CAC TTC AAC CAC AGT GGC ATC TGT GAG CTG CAC TGC CCA GCC CTG GTC     816
His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
            260                 265                 270

ACC TAC AAC ACA GAC ACG TTT GAG TCC ATG CCC AAT CCC GAG GGC CGG     864
Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
        275                 280                 285

TAT ACA TTC GGC GCC AGC TGT GTG ACT GCC TGT CCC TAC AAC TAC CTT     912
Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
    290                 295                 300
```

Fig. 15 (SEQ ID NO: 9)

```
TCT ACG GAC GTG GGA TCC TGC ACC CTC GTC TGC CCC CTG CAC AAC CAA      960
Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
305                 310                 315                 320

GAG GTG ACA GCA GAG GAT GGA ACA CAG CGG TGT GAG AAG TGC AGC AAG     1008
Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
                325                 330                 335

CCC TGT GCC CGA GTG TGC TAT GGT CTG GGC ATG GAG CAC TTG CGA GAG     1056
Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
            340                 345                 350

GTG AGG GCA GTT ACC AGT GCC AAT ATC CAG GAG TTT GCT GGC TGC AAG     1104
Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
        355                 360                 365

AAG ATC TTT GGG AGC CTG GCA TTT CTG CCG GAG AGC TTT GAT GGG GAC     1152
Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
    370                 375                 380

CCA GCC TCC AAC ACT GCC CCG CTC CAG CCA GAG CAG CTC CAA GTG TTT     1200
Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385                 390                 395                 400

GAG ACT CTG GAA GAG ATC ACA GGT TAC CTA TAC ATC TCA GCA TGG CCG     1248
Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
                405                 410                 415

GAC AGC CTG CCT GAC CTC AGC GTC TTC CAG AAC CTG CAA GTA ATC CGG     1296
Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
            420                 425                 430

GGA CGA ATT CTG CAC AAT GGC GCC TAC TCG CTG ACC CTG CAA GGG CTG     1344
Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
        435                 440                 445

GGC ATC AGC TGG CTG GGG CTG CGC TCA CTG AGG GAA CTG GGC AGT GGA     1392
Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
    450                 455                 460

CTG GCC CTC ATC CAC CAT AAC ACC CAC CTC TGC TTC GTG CAC ACG GTG     1440
Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
465                 470                 475                 480
```

Fig. 15 (SEQ ID NO: 9)

```
ATC CTC ATC AAG CGA CGG CAG CAG AAG ATC CGG AAG TAC ACG ATG CGG      2064
Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
        675                 680                 685

AGA CTG CTG CAG GAA ACG GAG CTG GTG GAG CCG CTG ACA CCT AGC GGA      2112
Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly
    690                 695                 700

GCG ATG CCC AAC CAG GCG CAG ATG CGG ATC CTG AAA GAG ACG GAG CTG      2160
Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu
705                 710                 715                 720

AGG AAG GTG AAG GTG CTT GGA TCT GGC GCT TTT GGC ACA GTC TAC AAG      2208
Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
                725                 730                 735

GGC ATC TGG ATC CCT GAT GGG GAG AAT GTG AAA ATT CCA GTG GCC ATC      2256
Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
            740                 745                 750

AAA GTG TTG AGG GAA AAC ACA TCC CCC AAA GCC AAC AAA GAA ATC TTA      2304
Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
        755                 760                 765

GAC GAA GCA TAC GTG ATG GCT GGT GTG GGC TCC CCA TAT GTC TCC CGC      2352
Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
    770                 775                 780

CTT CTG GGC ATC TGC CTG ACA TCC ACG GTG CAG CTG GTG ACA CAG CTT      2400
Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
785                 790                 795                 800

ATG CCC TAT GGC TGC CTC TTA GAC CAT GTC CGG GAA AAC CGC GGA CGC      2448
Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
                805                 810                 815

CTG GGC TCC CAG GAC CTG CTG AAC TGG TGT ATG CAG ATT GCC AAG GGG      2496
Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
            820                 825                 830

ATG AGC TAC CTG GAG GAT GTG CGG CTC GTA CAC AGG GAC TTG GCC GCT      2544
Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
        835                 840                 845
```

Fig. 15 (SEQ ID NO: 9)

CGG AAC GTG CTG GTC AAG AGT CCC AAC CAT GTC AAA ATT ACA GAC TTC 2592
Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
850 855 860

GGG CTG GCT CGG CTG CTG GAC ATT GAC GAG ACA GAG TAC CAT GCA GAT 2640
Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
865 870 875 880

GGG GGC AAG GTG CCC ATC AAG TGG ATG GCG CTG GAG TCC ATT CTC CGC 2688
Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
885 890 895

CGG CGG TTC ACC CAC CAG AGT GAT GTG TGG AGT TAT GGT GTG ACT GTG 2736
Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
900 905 910

TGG GAG CTG ATG ACT TTT GGG GCC AAA CCT TAC GAT GGG ATC CCA GCC 2784
Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
915 920 925

CGG GAG ATC CCT GAC CTG CTG GAA AAG GGG GAG CGG CTG CCC CAG CCC 2832
Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
930 935 940

CCC ATC TGC ACC ATT GAT GTC TAC ATG ATC ATG GTC AAA TGT TGG ATG 2880
Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
945 950 955 960

ATT GAC TCT GAA TGT CGG CCA AGA TTC CGG GAG TTG GTG TCT GAA TTC 2928
Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
965 970 975

TCC CGC ATG GCC AGG GAC CCC CAG CGC TTT GTG GTC ATC CAG AAT GAG 2976
Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu
980 985 990

GAC TTG GGC CCA GCC AGT CCC TTG GAC AGC ACC TTC TAC CGC TCA CTG 3024
Asp Leu Gly Pro Ala Ser Pro Leu Asp Ser Thr Phe Tyr Arg Ser Leu
995 1000 1005

CTG GAG GAC GAT GAC ATG GGG GAC CTG GTG GAT GCT GAG GAG TAT CTG 3072
Leu Glu Asp Asp Asp Met Gly Asp Leu Val Asp Ala Glu Glu Tyr Leu
1010 1015 1020

Fig. 15 (SEQ ID NO: 9)

```
GTA CCC CAG CAG GGC TTC TTC TGT CCA GAC CCT GCC CCG GGC GCT GGG     3120
Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro Ala Pro Gly Ala Gly
1025                1030                1035                1040

GGC ATG GTC CAC CAC AGG CAC CGC AGC TCA TCT ACC AGG AGT GGC GGT     3168
Gly Met Val His His Arg His Arg Ser Ser Ser Thr Arg Ser Gly Gly
                1045                1050                1055

GGG GAC CTG ACA CTA GGG CTG GAG CCC TCT GAA GAG GAG GCC CCC AGG     3216
Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu Ala Pro Arg
            1060                1065                1070

TCT CCA CTG GCA CCC TCC GAA GGG GCT GGC TCC GAT GTA TTT GAT GGT     3264
Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser Asp Val Phe Asp Gly
        1075                1080                1085

GAC CTG GGA ATG GGG GCA GCC AAG GGG CTG CAA AGC CTC CCC ACA CAT     3312
Asp Leu Gly Met Gly Ala Ala Lys Gly Leu Gln Ser Leu Pro Thr His
    1090                1095                1100

GAC CCC AGC CCT CTA CAG CGG TAC AGT GAG GAC CCC ACA GTA CCC CTG     3360
Asp Pro Ser Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Val Pro Leu
1105                1110                1115                1120

CCC TCT GAG ACT GAT GGC TAC GTT GCC CCC CTG ACC TGC AGC CCC CAG     3408
Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu Thr Cys Ser Pro Gln
                1125                1130                1135

CCT GAA TAT GTG AAC CAG CCA GAT GTT CGG CCC CAG CCC CCT TCG CCC     3456
Pro Glu Tyr Val Asn Gln Pro Asp Val Arg Pro Gln Pro Pro Ser Pro
            1140                1145                1150

CGA GAG GGC CCT CTG CCT GCT GCC CGA CCT GCT GGT GCC ACT CTG GAA     3504
Arg Glu Gly Pro Leu Pro Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu
        1155                1160                1165

AGG CCC AAG ACT CTC TCC CCA GGG AAG AAT GGG GTC GTC AAA GAC GTT     3552
Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val
    1170                1175                1180

TTT GCC TTT GGG GGT GCC GTG GAG AAC CCC GAG TAC TTG ACA CCC CAG     3600
Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu Tyr Leu Thr Pro Gln
1185                1190                1195                1200
```

Fig. 15 (SEQ ID NO: 9)

```
GGA GGA GCT GCC CCT CAG CCC CAC CCT CCT CCT GCC TTC AGC CCA GCC      3648
Gly Gly Ala Ala Pro Gln Pro His Pro Pro Pro Ala Phe Ser Pro Ala
                1205                1210                1215

TTC GAC AAC CTC TAT TAC TGG GAC CAG GAC CCA CCA GAG CGG GGG GCT      3696
Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Pro Glu Arg Gly Ala
            1220                1225                1230

CCA CCC AGC ACC TTC AAA GGG ACA CCT ACG GCA GAG AAC CCA GAG TAC      3744
Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala Glu Asn Pro Glu Tyr
        1235                1240                1245

CTG GGT CTG GAC GTG CCA GTG TGA                                      3768
Leu Gly Leu Asp Val Pro Val
    1250                1255
```

Fig. 16 (SEQ ID NO: 10)

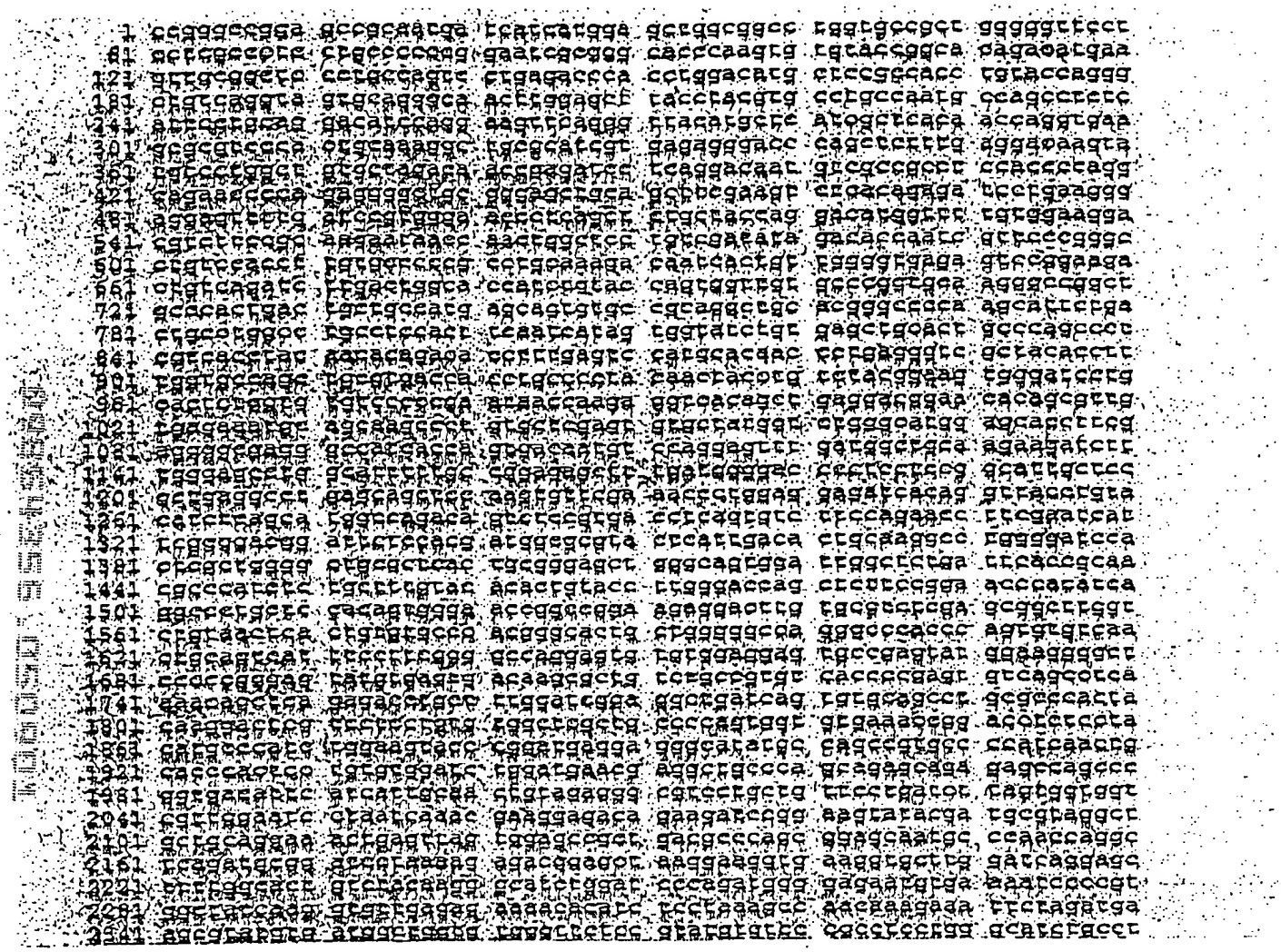
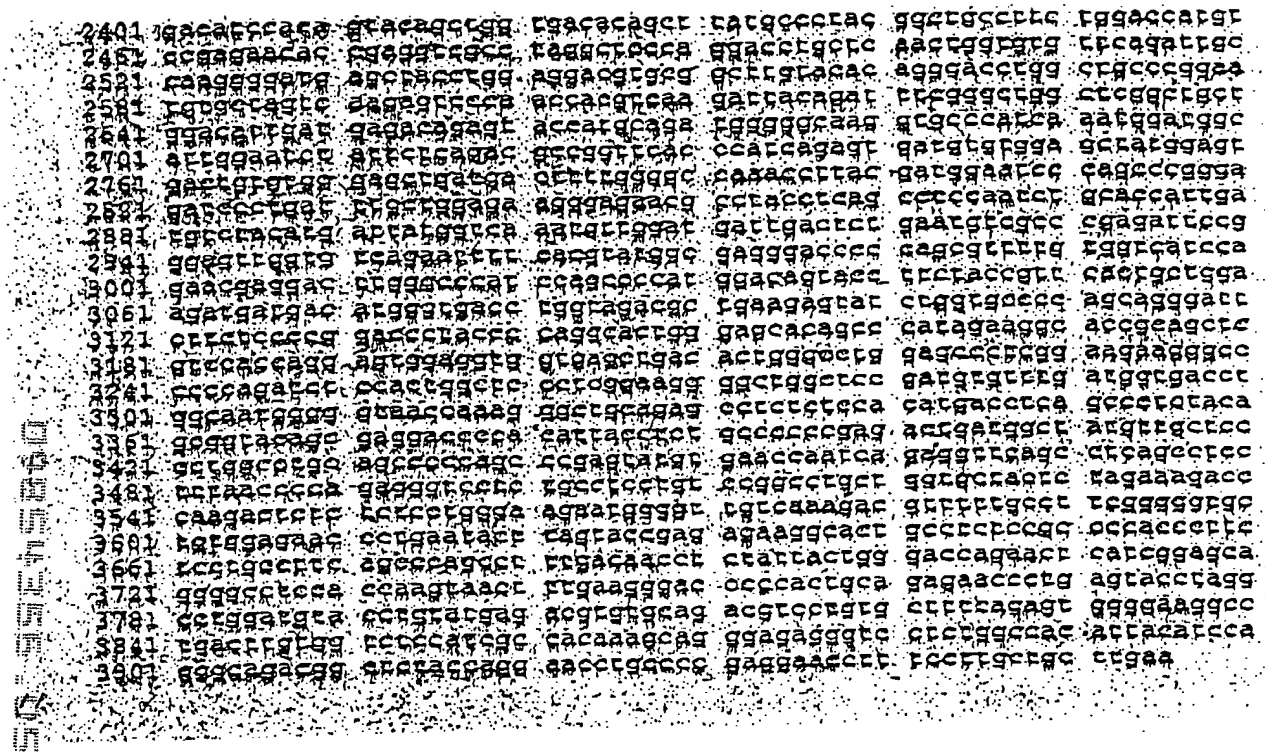


Fig. 16 (SEQ ID NO: 10)



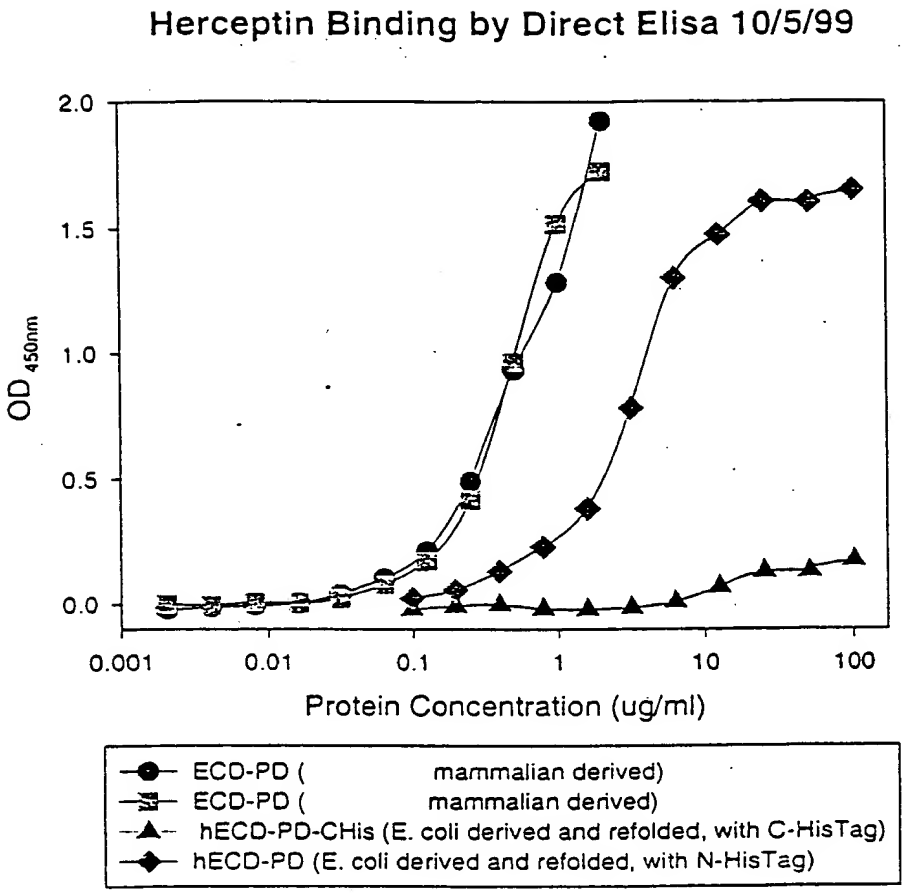
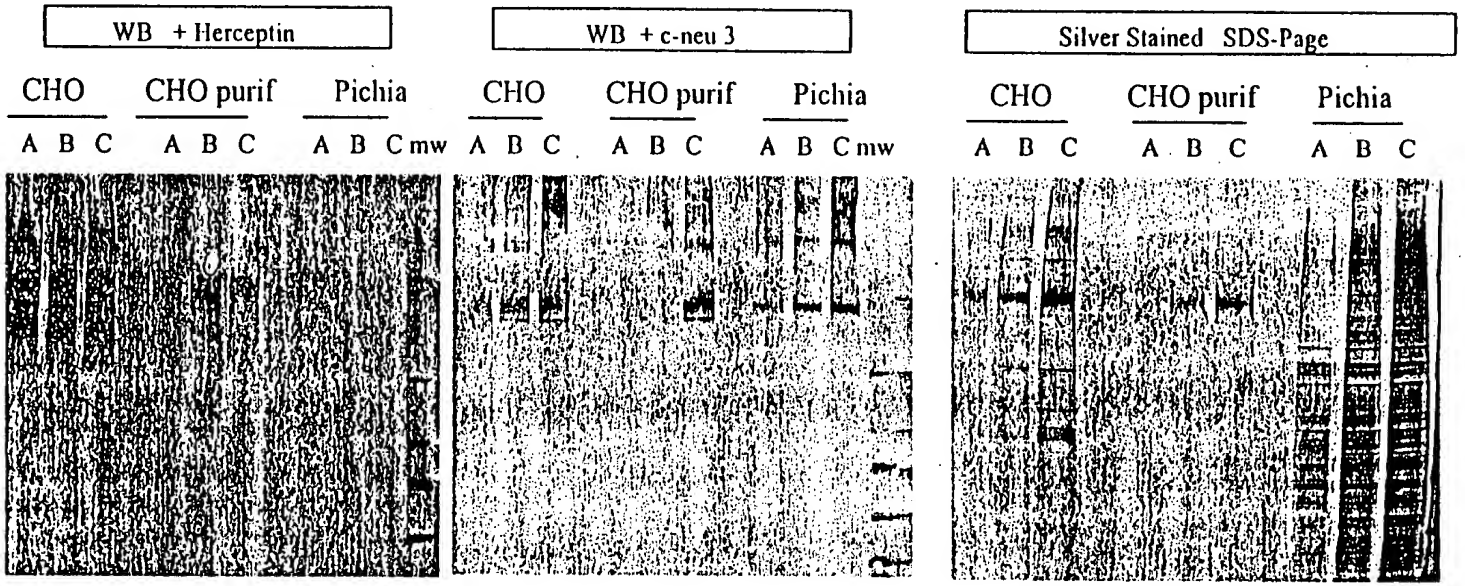

Herceptin Binding by Direct Elisa 10/5/99
2.0
1.5
1.0
0.5
0.0
OD 450nm
0.001
0.01
0.1
1
10
100
Protein Concentration (ug/ml)
ECD-PD (mammalian derived)
ECD-PD (mammalian derived)
hECD-PD-CHis (E. coli derived and refolded, with C-HisTag)
hECD-PD (E. coli derived and refolded, with N-HisTag)

Fig. 17

Fig. 18

Comparaison of Her2neu ECD-PD Expression in CHO-K1 (S/SF) and Pichia (Non reducing conditions)



Legend : CHO; A, B , C = 2,5µl / 5µl / 10µl

CHO purif; A , B , C = 125ng / 250ng / 500ng

Pichia ; A ,B ,C = 2,5µl / 5µl /10µl from a 1/30 dilution of OD 120

Fig. 19 (SEQ ID NO:11)

```
atggagctgg cggcctggtg ccgttggggg ttcctcctcg ccctcctgtc ccccggagcc      60
gcgggtaccc aagtgtgtac cggtaccgac atgaagttgc gactccctgc cagtcctgag     120
acccacctgg acatgcttcg ccacctctac cagggctgtc aggtggtgca gggcaatttg     180
gagcttacct acctgcccgc caatgccagc ctctcattcc tgcaggacat ccaggaagtc     240
cagggataca tgctcatcgc tcacaaccga gtgaaacacg tcccactgca gaggttgcgc     300
atcgtgagag ggactcagct ctttgaggac aagtatgccc tggctgtgct agacaaccga     360
gacccttttgg acaacgtcac caccgccgcc ccaggcagaa ccccagaagg gctgcgggag     420
ctgcagcttc gaagtctcac agagatcttg aagggaggag ttttgatccg tgggaaccct     480
cagctctgct accaggacat ggttttgtgg aaggatgtcc tccgtaagaa taaccagctg     540
gctcctgtcg acatggacac caatcgttcc cgggcctgtc caccttgtgc cccaacctgc     600
aaagacaatc actgttgggg tgagagtcct gaagactgtc agatcttgac tggcaccatc     660
tgtactagtg gctgtgcccg gtgcaagggc cggctgccca ctgactgttg ccatgagcag     720
tgtgctgcag gctgcacggg tcccaagcat tctgactgcc tggcctgcct ccacttcaat     780
catagtggta tctgtgagct gcactgcccg gccctcatca cctacaacac agacaccttc     840
gagtccatgc tcaaccctga gggtcgctac acctttggtg ccagctgtgt gaccacctgc     900
ccctacaact acctctccac ggaagtggga tcctgcactc tggtctgtcc cccgaacaac     960
caagaggtca cagctgagga cggaacacag cggtgtgaga aatgcagcaa gccctgtgct    1020
ggagtatgct atggtctggg catggagcac ctccgagggg cgagggccat caccagtgac    1080
aatatccagg agtttgctgg ctgcaagaag atctttggga gcctggcatt tttgccggag    1140
agctttgatg ggaacccctc ctccggcgtt gccccactga agccagagca tctccaagtg    1200
ttcgaaaccc tggaggagat cacaggttac ctatacattt cagcatggcc agagagcttc    1260
caagacctca gtgtcttcca gaaccttcgg gtcattcggg gacggattct ccatgatggt    1320
gcttactcat tgacgttgca aggcctgggg attcactcac tggggctacg ctcactgcgg    1380
gagctgggca gtggattggc tctcattcac cgcaacaccc atctctgctt tgtaaacact    1440
gtaccttggg accagctctt ccggaacccg caccaggccc tactccacag tgggaaccgg    1500
ccagaagagg catgtggtct tgagggcttg gtctgtaact cactgtgtgc ccgtgggcac    1560
tgctgggggc cagggcccac ccagtgtgtc aactgcagtc agttcctccg gggccaggag    1620
tgtgtggagg agtgccgagt atggaagggg ctccccaggg agtatgtgag gggcaagcac    1680
tgtctgccat gccaccccga gtgtcagcct caaaacagct cggagacctg ctatggatcg    1740
gaggctgacc agtgtgaggc ttgtgcccac tacaaggact catcttcctg tgtggctcgc    1800
tgccccagtg gtgtgaagcc agacctctcc tacatgccta tctggaagta cccggatgag    1860
gagggcatat gtcagccatg ccccatcaac tgcacccact catgtgtgga cctggacgaa    1920
cgaggctgcc cagcagagca gagagccagc ccagtgacat tcatcattgc aactgtggtg    1980
ggcgtcctgt tgttcctgat catagtggtg gtcattggaa tcctaatcaa acgaaggcga    2040
cagaagatcc ggaagtatac catgcgtagg ctgctgcagg agaccgagct ggtggagccg    2100
ctgacgccca gtggagctgt gcccaaccag gctcagatgc ggatcctaaa ggagacagag    2160
ctaaggaagc tgaaggtgct tgggtcagga gccttcggca ctgtctacaa gggcatctgg    2220
atcccagatg gggagaacgt gaaaatcccc gtggccatca aggtgttgag ggaaaacaca    2280
tctcctaaag ctaacaaaga aatcctagat gaagcgtacg tcatggctgg tgtgggttct    2340
ccatatgtgt cccgcctcct gggcatctgc ctgacatcca cagtgcagct ggtgacacag    2400
cttatgccct atggctgcct tctggaccat gtccgagaac accgaggtcg cttaggctcc    2460
caggacctgc tcaactggtg tgttcagatt gccaagggga tgagctacct ggaggaagtt    2520
cggcttgttc acagggacct agctgcccga aacgtgctag tcaagagtcc caaccacgtc    2580
aagattaccg acttcgggct ggcacggctg ctggacattg atgagactga ataccatgca    2640
gatgggggca aggtgcccat caagtggatg gcattggaat ctattctcag acgccggttc    2700
actcatcaga gtgatgtgtg gagctatggt gtgactgtgt gggagctgat gacctttggg    2760
gccaaacctt acgatgggat cccagctcgg gagatccctg atttgctgga gaagggagaa    2820
cgcctacctc agcctccaat ctgcaccatc gacgtctaca tgatcatggt caaatgttgg    2880
atgattgact ccgaatgtcg cccgagattc cgggagttgg tatcagaatt ctcccgtatg    2940
gcaagggacc cccagcgctt tgtggtcatc cagaacgagg acttaggccc ctccagcccc    3000
atggacagca ccttctaccg ttcactgctg gaggatgatg acatggggga gctggtcgat    3060
gctgaagagt acctggtacc ccagcaggga ttcttctccc cagaccctgc cctaggtact    3120
gggagcacag cccaccgcag acaccgcagc tcgtcggcca ggagtggcgg tggtgagctg    3180
acactgggcc tggagccctc ggaagaagag ccccccagat ctccactggc tccctccgaa    3240
ggggctggct ccgatgtgtt tgatggtgac ctggcagtgg gggtaaccaa aggactgcag    3300
agcctctctc cacatgacct cagccctcta cagcggtaca gtgaggatcc cacattacct    3360
```

Fig. 19 (SEQ ID NO:11)

```
ctgcccccg agactgatgg ctacgttgct cccctggcct gcagcccccca gcccgagtat     3420
gtgaaccagc cagaggttcg gcctcagtct cccttgaccc cagagggtcc tccgcctccc     3480
atccgacctg ctggtgctac tctagaaaga cccaagactc tctctcctgg gaaaaatggg     3540
gttgtcaaag acgtttttgc ctttgggggt gctgtggaga accctgaata cctagcaccc     3600
agagcaggca ctgcctctca gccccaccct tctcctgcct tcagcccagc ctttgacaac     3660
ctctattact gggaccagaa ctcatcggag cagggtcctc caccaagtac ctttgaaggg     3720
acccccactg cagagaaccc tgagtaccta ggcctggatg tgccagtatg a              3771
```

Fig. 20 (SEQ ID NO:14)

```
Met Glu Leu Ala Ala Trp Cys Arg Trp Gly Phe Leu Leu Ala Leu Leu
1               5                   10                  15
Ser Pro Gly Ala Ala Gly Thr Gln Val Cys Thr Gly Thr Asp Met Lys
            20                  25                  30
Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
        35                  40                  45
Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
    50                  55                  60
Leu Pro Ala Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65                  70                  75                  80
Gln Gly Tyr Met Leu Ile Ala His Asn Arg Val Lys His Val Pro Leu
                85                  90                  95
Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Lys Tyr
            100                 105                 110
Ala Leu Ala Val Leu Asp Asn Arg Asp Pro Leu Asp Asn Val Thr Thr
        115                 120                 125
Ala Ala Pro Gly Arg Thr Pro Glu Gly Leu Arg Glu Leu Gln Leu Arg
    130                 135                 140
Ser Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Arg Gly Asn Pro
145                 150                 155                 160
Gln Leu Cys Tyr Gln Asp Met Val Leu Trp Lys Asp Val Leu Arg Lys
                165                 170                 175
Asn Asn Gln Leu Ala Pro Val Asp Met Asp Thr Asn Arg Ser Arg Ala
            180                 185                 190
Cys Pro Pro Cys Ala Pro Thr Cys Lys Asp Asn His Cys Trp Gly Glu
        195                 200                 205
Ser Pro Glu Asp Cys Gln Ile Leu Thr Gly Thr Ile Cys Thr Ser Gly
    210                 215                 220
Cys Ala Arg Cys Lys Gly Arg Leu Pro Thr Asp Cys Cys His Glu Gln
225                 230                 235                 240
Cys Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys
                245                 250                 255
Leu His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu
            260                 265                 270
Ile Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Leu Asn Pro Glu Gly
        275                 280                 285
Arg Tyr Thr Phe Gly Ala Ser Cys Val Thr Thr Cys Pro Tyr Asn Tyr
    290                 295                 300
Leu Ser Thr Glu Val Gly Ser Cys Thr Leu Val Cys Pro Pro Asn Asn
305                 310                 315                 320
Gln Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser
                325                 330                 335
Lys Pro Cys Ala Gly Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg
            340                 345                 350
Gly Ala Arg Ala Ile Thr Ser Asp Asn Ile Gln Glu Phe Ala Gly Cys
        355                 360                 365
Lys Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly
    370                 375                 380
Asn Pro Ser Ser Gly Val Ala Pro Leu Lys Pro Glu His Leu Gln Val
385                 390                 395                 400
Phe Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp
                405                 410                 415
Pro Glu Ser Phe Gln Asp Leu Ser Val Phe Gln Asn Leu Arg Val Ile
```

Fig. 20 (SEQ ID NO:14)

```
                420                     425                     430
Arg Gly Arg Ile Leu His Asp Gly Ala Tyr Ser Leu Thr Leu Gln Gly
            435                     440                     445
Leu Gly Ile His Ser Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser
        450                     455                     460
Gly Leu Ala Leu Ile His Arg Asn Thr His Leu Cys Phe Val Asn Thr
465                     470                     475                     480
Val Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His
                    485                     490                     495
Ser Gly Asn Arg Pro Glu Glu Ala Cys Gly Leu Glu Gly Leu Val Cys
                500                     505                     510
Asn Ser Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln
            515                     520                     525
Cys Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu
        530                     535                     540
Cys Arg Val Trp Lys Gly Leu Pro Arg Glu Tyr Val Arg Gly Lys His
545                     550                     555                     560
Cys Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Ser Ser Glu Thr
                    565                     570                     575
Cys Tyr Gly Ser Glu Ala Asp Gln Cys Glu Ala Cys Ala His Tyr Lys
                580                     585                     590
Asp Ser Ser Ser Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp
            595                     600                     605
Leu Ser Tyr Met Pro Ile Trp Lys Tyr Pro Asp Glu Glu Gly Ile Cys
        610                     615                     620
Gln Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Glu
625                     630                     635                     640
Arg Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Val Thr Phe Ile Ile
                    645                     650                     655
Ala Thr Val Val Gly Val Leu Leu Phe Leu Ile Ile Val Val Val Ile
                660                     665                     670
Gly Ile Leu Ile Lys Arg Arg Arg Gln Lys Ile Arg Lys Tyr Thr Met
            675                     680                     685
Arg Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser
        690                     695                     700
Gly Ala Val Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu
705                     710                     715                     720
Leu Arg Lys Leu Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr
                    725                     730                     735
Lys Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala
                740                     745                     750
Ile Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile
            755                     760                     765
Leu Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser
        770                     775                     780
Arg Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln
785                     790                     795                     800
Leu Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu His Arg Gly
                    805                     810                     815
Arg Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Val Gln Ile Ala Lys
                820                     825                     830
Gly Met Ser Tyr Leu Glu Glu Val Arg Leu Val His Arg Asp Leu Ala
            835                     840                     845
Ala Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp
        850                     855                     860
Phe Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala
865                     870                     875                     880
```

Fig. 20 (SEQ ID NO:14)

Asp Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu
885 890 895
Arg Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr
900 905 910
Val Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro
915 920 925
Ala Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln
930 935 940
Pro Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp
945 950 955 960
Met Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu
965 970 975
Phe Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn
980 985 990
Glu Asp Leu Gly Pro Ser Ser Pro Met Asp Ser Thr Phe Tyr Arg Ser
995 1000 1005
Leu Leu Glu Asp Asp Asp Met Gly Glu Leu Val Asp Ala Glu Glu Tyr
1010 1015 1020
Leu Val Pro Gln Gln Gly Phe Phe Ser Pro Asp Pro Ala Leu Gly Thr
1025 1030 1035 1040
Gly Ser Thr Ala His Arg Arg His Arg Ser Ser Ser Ala Arg Ser Gly
1045 1050 1055
Gly Gly Glu Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu Pro Pro
1060 1065 1070
Arg Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser Asp Val Phe Asp
1075 1080 1085
Gly Asp Leu Ala Val Gly Val Thr Lys Gly Leu Gln Ser Leu Ser Pro
1090 1095 1100
His Asp Leu Ser Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Leu Pro
1105 1110 1115 1120
Leu Pro Pro Glu Thr Asp Gly Tyr Val Ala Pro Leu Ala Cys Ser Pro
1125 1130 1135
Gln Pro Glu Tyr Val Asn Gln Pro Glu Val Arg Pro Gln Ser Pro Leu
1140 1145 1150
Thr Pro Glu Gly Pro Pro Pro Pro Ile Arg Pro Ala Gly Ala Thr Leu
1155 1160 1165
Glu Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp
1170 1175 1180
Val Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu Tyr Leu Ala Pro
1185 1190 1195 1200
Arg Ala Gly Thr Ala Ser Gln Pro His Pro Ser Pro Ala Phe Ser Pro
1205 1210 1215
Ala Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asn Ser Ser Glu Gln Gly
1220 1225 1230
Pro Pro Pro Ser Thr Phe Glu Gly Thr Pro Thr Ala Glu Asn Pro Glu
1235 1240 1245
Tyr Leu Gly Leu Asp Val Pro Val
1250 1255